

FOOD PRODUCT COMPRISING AN EXTRACT FROM PINE NEEDLES

This invention relates to a food product, in particular a foodstuff comprising a fat continuous emulsion.

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Fat continuous emulsions are contained in a number of foodstuffs, including margarines and low fat spreads. Low fat spreads are disclosed in US 4917915, for example. The fat phase is responsible for at least part of the oral properties of the foodstuffs. Certain low fat spreads are marketed for their health properties, including 10 the ability to reduce cholesterol.

WO 98/28990 discloses a method of preparing food seasonings, food ingredients and food items using plant sterols and/or stanols together with raised levels of one or more of magnesium, calcium and potassium. Ingestion of the food is said to lead to a 15 decrease in both cholesterol level and blood pressure.

There remains a need for food products comprising a fat continuous emulsion that have improved properties, including butter-like appearance and oral properties. There is also a need for food products of this type that have other health benefits and/or 20 improved health properties.

Pine needles are the leaves of plants of the Pinaceae family, including the genus *Pinus*. Certain types of pine needles are available in abundant supply and have been used for various purposes. Pine needle extracts have been described as being useful 25 in specific beverages in JP 08107778 A and JP 07059538. Rice cakes containing pine needle extracts are described in JP 01218562 A.

A process for the extraction of taxol from pine needles is described in WO 94/15483.

High blood pressure (or hypertension) is known to be associated with many medical problems. High blood pressure directly increases the risk of coronary heart disease and stroke. High blood pressure most predominantly occurs in people over 35 years old but environmental and genetic factors and some medical conditions, such as 5 diabetes mellitus, gout or kidney disease can lead to an increased risk of high blood pressure in people of all ages.

US 6,329,000 discloses the use of certain pine needle extracts for treating various diseases including myocarditis, angina, arrhythmia, diabetes, senile dementia, sudden 10 deafness and hypertension. The pine needle extracts are obtained by a relatively simple extraction using water and alcohol as solvents.

US 5,607,971 discloses the extraction of vasoactive lipids from *Pinus ponderosa* using methanol, diethyl ether and methylene chloride. The compounds isolated are 15 esterified alkanediols.

US 5,690,984 describes a beverage made from pine needles by boiling the needles in water at high pressure in a pressure vessel together with a mixture of other natural products.

20 Pine needles and their extracts may contain isocupressic acids. Isocupressic acids have been described as causing toxicity problems in beef cattle. It has been found that in US 6,329,000, US 5,607,971 and US 5,690,984, a substantial amount of isocupressic acid remains in the extracts.

25 US 5,466,453 teaches a method for improving the taste of a pine extract. US 6,254,858 relates to a hair treatment composition containing, amongst other components, pine juices. US 187,802 describes a disinfecting composition containing maple sugar and extracts of pine needles and juniper berries. The inventors believe 30 that the products will contain substantial amounts of isocupressic acid.

CN-A-1102111 describes the preparation of raw pine needle juice.

Pine needle extracts have now been found to be useful for incorporation into food  
5 products comprising a fat continuous emulsion.

According to the invention, there is provided a food product comprising a fat  
continuous emulsion, wherein the emulsion comprises a material comprising one or  
more organic compounds, said material being obtainable as an extract from pine  
10 needles.

The extract is preferably an aqueous extract. When the extract is an aqueous extract,  
the food product comprises an aqueous phase and the material is preferably present in  
the aqueous phase.

15 The material preferably comprises at least 2 components A and B, wherein A is a  
compound that is obtainable from a mixture of A and B, such as pine needles, by  
elution from a silica column using 100 % methanol as eluent and B is a compound  
obtainable from the same silica column using methanol/water mixtures (5-40 % by  
20 volume) in a series of subsequent elutions. A is preferably selected from the group  
consisting of phytosterol, polyphenols, bioflavonoids, tannins, organic acids and their  
complexes. B is preferably selected from the group consisting of amino acids,  
peptides, proteins, quercetin, terpenoids, flavonol glycosides, biflavones,  
proanthocyanidins, polyprenols, lignans and minerals. The material may comprise  
25 one or more compounds A and one or more compounds B. Preferably, the material  
comprises A (or total A compounds where more than one A compound is present) in  
an amount of from 5 to 60 wt %, preferably 10 to 50 wt%, most preferably 15 to 40  
wt%, and the material comprises B (or total B compounds where more than one B  
compound is present) in an amount of from 1 to 15 wt %, preferably 2 to 12wt %,  
30 most preferably 3 to 10 wt%, based on the weight of the material.

Therefore, in one embodiment, the material comprises at least one compound selected from the group consisting of phytosterol, polyphenols, bioflavonoids, tannins, organic acids and their complexes and at least one compound selected from the group 5 consisting of amino acids, peptides, proteins, quercetin, terpenoids, flavonol glycosides, biflavones, proanthocyanidins, polyprenols, lignans and minerals.

The material preferably contains isocupressic acid compounds in an amount of less than 0.005 wt%, more preferably less than 0.003 wt%, even more preferably less than 10 0.002 wt% such as less than 0.001 wt%. The terms "isocupressic acid compounds" and "isocupressic acids" are used synonymously herein and refer to isocupressic acid itself and and preferably related diterpene acids found in pine needles and their extracts, such as imbricatoloic acid, agathic acid, dihydroagathic acid and tetrahydroagathic acid. Isocupressic acids may be in the form of derivatives of these 15 acids, for example, acetyl imbricatoloic acid and acetyl isocupressic acid. Preferably, therefore, the material of the invention contains isocupressic acid, imbricatoloic acid, agathic acid, dihydroagathic acid, tetrahydroagathic acid, acetyl imbricatoloic acid and acetyl isocupressic acid in an amount of less than 0.005 wt%, more preferably less than 0.003 wt%, even more preferably less than 0.002 wt% such as less than 0.001 20 wt%. Preferably, the material is free of isocupressic acids or substantially free of isocupressic acids (i.e., to the extent that the presence of isocupressic acids cannot be detected by conventional techniques and/or has no effect on the properties of the composition). The level of isocupressic acids can be determined, for example, by GCMS.

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The material is obtainable, and is preferably obtained, from pine needles. Pine needles are preferably from species of pine other than *Pinus ponderosa*. Pine species include *Pinus albicaulis*, *Pinus aristata*, *Pinus attenuata*, *Pinus balfouriana*, *Pinus banksiana*, *Pinus bungeana*, *Pinus cembra*, *Pinus cembroides*, *Pinus clausa*, *Pinus contorta*, *Pinus coulteri*, *Pinus densiflora*, *Pinus echinata*, *Pinus edulis*, *Pinus* 30

*elliottii, Pinus engelmannii, Pinus flexilis, Pinus glabra, Pinus heldreichii, Pinus jeffreyi, Pinus lambertiana, Pinus longaeva, Pinus massoniana, Pinus monophylla, Pinus monticola, Pinus mugo, Pinus muricata, Pinus nigra, Pinus palustris, Pinus parviflora, Pinus pungens, Pinus quadrifolia, Pinus radiata, Pinus resinosa, Pinus rigida, Pinus sabiniana, Pinus serotina, Pinus strobus, Pinus sylvestris, Pinus tabulaeformis, Pinus taeda, Pinus thunbergiana, Pinus torreyana, Pinus virginiana, Pinus yuannensis and Pinus washoensis.* Preferably, the material is from *Pinus massoniana, Pinus tabulaeformis or Pinus yuannensis*, more preferably, the material is from *Pinus massoniana*. The material preferably comprises one or 10 more organic compounds, more preferably two or more organic compounds. Organic compounds are compounds that comprise carbon, hydrogen and oxygen atoms and optionally other atoms such as nitrogen, phosphorus and sulphur.

Materials of the invention preferably comprise shikimic acid and/or quinic acid. 15 Shikimic acid is preferably present in the materials of the invention in an amount by weight of the material of at least 10 %, preferably at least 12 %, more preferably at least 15 %, such as at least 16 %, at least 17 %, at least 18 %, at least 19 % or at least 20 %. The upper limit for the amount of shikimic acid is typically no more than 50 %, more preferably no more than 40 %, such as no more than 30 %, or no more than 20 25 %, again by weight of the material. The shikimic acid may be present as a salt or other derivative, such as an acetyl ester.

Quinic acid (typically as D-quinic acid) is preferably present in the materials of the invention in an amount by weight of the material of at least 5 %, preferably at least 6 25 %, more preferably at least 7 %, such as at least 8 %, at least 9 %, at least 10 %, at least 12 % or at least 15 %. The upper limit for the amount of quinic acid is typically no more than 30 %, more preferably no more than 27 %, such as no more than 25 %,

or no more than 20 %, again by weight of the material. The quinic acid may be present as a salt or other derivative, such as an acetyl ester.

Specific preferred materials of the invention comprise shikimic acid and quinic acid  
5 in the amounts specified in the preceding two paragraphs. An example of such a material comprises at least 10 % by weight shikimic acid and at least 5 % by weight quinic acid, such as 10 % to 30 % shikimic acid and 5 % to 20 % quinic acid.

Materials of the invention may further comprise low molecular weight sugars,  
10 preferably sugars having a molecular weight below 1000 Daltons. Preferred low molecular weight sugars are monosaccharide units and include glucose, fructose and inositol. The amount of low molecular weight sugars is preferably from 15 % to 50 % by weight, such as 20 % to 40 %, or 20 % to 35 % by weight of the material.

15 The material is preferably obtainable by a process which comprises the following steps:

treating pine needles with a solvent selected from water, organic solvents and mixtures thereof (preferably water), preferably at an elevated temperature of from 40 °C to 110 °C, to form a first extract;

20 removing isocupressic acid compounds from the first extract, preferably by treatment with an ion exchange resin and/or activated carbon (preferably whilst the first extract is in aqueous solution, more preferably at an elevated temperature); and optionally, filtering and concentrating the treated extract to obtain the material as a powder or a concentrate. Preferably, prior to step (a), the pine needles are pretreated  
25 with a non-polar solvent (e.g., an alkane having from 4 to 10 carbon atoms, such as hexane), more preferably at a temperature of from 40 °C to 90 °C. This pretreatment typically removes at least a part of the isocupressic acids.

The food product can consist of, or consist essentially of, the fat continuous emulsion (e.g., in the case of certain margarines or low fat spreads). Thus, the fat continuous emulsion can constitute all of the food product. Alternatively, the food product can 5 contain the fat continuous emulsion together with one or more other food ingredients or additives.

The fat continuous emulsion preferably has a fat content of from 0.5 to 99.5 wt%, preferably 20 to 85 wt%, most preferably 30 to 80 wt%, based on the weight of the 10 food product. Water is preferably present in the food product in an amount of from 0.5 to 99.5 wt%, more preferably 10 wt% to 50 wt%. The food product thus preferably comprises an aqueous phase and a fat phase.

The solid fat content of the fat in the fat continuous emulsion can conveniently be 15 determined by measuring the NMR N-value as described in Fette, Seifen, Anstrichmittel, 80 (1978), 180-186, which indicates the amount of fat present in the solid state expressed in percentage of the weight of the fat. The fat phase preferably displays a solid fat content (measured by NMR on a non-stabilised fat) at 5°C (=N5) of >10, more preferably >20, and a solid fat content at 35°C (=N35) of <20, 20 preferably <10, most preferably <5. Non-stabilised means that the N-value is measured after first melting the fat above 80° C, whereupon the melt is cooled to 0° C and kept at 0° C for 30 minutes, then the fat is heated to the measurement temperature and kept at that temperature for 30 minutes, whereupon the N-value is measured.

25 One embodiment of the invention is a food product comprising fat and water and having a fat content of from 0.5 to 99.5 wt %, wherein the fat phase comprises at least two components (D) and (E), (D) having an N20>20 and (E) having a content of mono- and di-unsaturated fatty acid residues of at least 25 wt%. Component (D) is preferably selected from the group consisting of palm fractions, interesterified 30 hardened palm oil and hardened palm kernel oil and fractions thereof, interesterified

mixtures of liquid oils and hardened liquid oils, interesterified fractions of palm kernel oil and palm oil, particularly palm kernel stearine and palm oil stearine, and fractions thereof, and fats containing at least 20 wt % of SUS triglycerides. Preferably, (E) has a content of mono- and di-unsaturated fatty acid residues of from 5 55 to 95 wt% and examples of component (E) include oils selected from the group consisting of sunflower oil, high oleic sunflower oil, rape seed oil, high oleic rape seed oil, palm oil olein, corn oil, soybean oil and high oleic soybean oil. The weight ratio of (D) to (E) is preferably from 10:1 to 1:20, more preferably from 1:1 to 1:15, even more preferably from 1:4 to 1:10.

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Preferably, the food products of the invention are essentially free of trans fatty acids (which are carboxylic acids containing from 12 to 24 carbon atoms and having one carbon-carbon double bond) i.e., they contain trans fatty acids in an amount of less than 1% by weight, preferably less than 0.5% by weight, more preferably less than 15 0.1% by weight, such as less than 0.05% or less than 0.01% by weight.

The food product preferably contains the material in an amount of from 0.01wt% to 20wt% (e.g., 0.01wt% to 1 wt%), more preferably from 0.05wt% to 15wt% (e.g., 0.05wt% to 1 wt%), even more preferably from 0.1wt% to 12wt% (e.g., 0.1wt% to 1 20 wt%), based on the total weight of the food product and on the weight of the dry material.

The food product of the invention preferably has one or more of the following properties compared to a corresponding product that does not contain the material: 25 increased hardness, improved texture, increased aeration, improved spreadability, improved oral properties, improved mouthfeel, improved flavour impact, better colour, improved viscosity, increased ease of processing and improved health properties. The properties are improved compared to an otherwise identical food product that does not contain the material. Preferred properties that are improved

according to the invention are oral properties and/or visual appearance, in particular increased similarity to butter in terms of oral properties and/or visual appearance.

Increased hardness of the food product is a particularly preferred advantage of the  
5 invention. The increased hardness is, for example, evident when the product is spread onto a substrate with a knife.

The material of the invention has therapeutic activity. The term "therapeutic activity" in this context means usefulness in the treatment, inhibition or prevention of diseases  
10 or disorders. Diseases and disorders include, but are not limited to, high blood pressure (hypertension). In one aspect, the invention provides a food product of the invention, for use to lower blood pressure in mammals, particularly humans.

The invention may involve one or more of the following effects: lowering blood  
15 pressure; lowering systolic and/or diastolic blood pressure; reducing heart rate; reducing sympathetic nerve activity; reducing the chance of coronary heart disease; reducing the chance of aneurisms; reducing the chance of strokes; improving blood circulation; improving the cardiovascular system; improving blood vessel health;  
20 providing part of a healthy lifestyle; improving the chance of healthy circulation; reducing the effect of aging on the blood vessels; reducing cardiac stress; and improving recovery time after exercising.

The invention also involves a method of lowering blood pressure (and/or treating  
25 hypertension) in a mammal which comprises the provision of an effective amount of a food product of the invention. The invention further contemplates the use of a food product of the invention in the manufacture of a composition for treating and/or preventing hypertension.

Preferred food products of the invention are margarines, low fat spreads, bakery spreads and cooking spreads. These products may be packaged and labelled for sale and are typically stored at low temperature (e.g., below 15°C). Preferred packaging includes containers with lids, preferably of plastics material and of a size sufficient to  
5 hold from 25 g to 1 kg of the food product.

Margarines and spreads can be made by using conventional techniques for the preparation of margarines, low fat spreads or very low fat spreads (less than 40wt% fat) (as disclosed for example in EP 089082, the contents of which are incorporated  
10 herein by reference).

A spread preferably has a continuous fat phase to give it suitable spreadability and to prevent microbiological deterioration. It should not release moisture when it is being spread. Furthermore, a spread should preferably be spreadable at refrigerator  
15 temperature (5°C), be stable at room temperature (20°C), but destabilize and release its flavour in the mouth.

The margarine or spread is a dispersion having a plastified continuous fat phase and a dispersed aqueous phase. As is common practice with respect to products of this  
20 kind, the term "continuous fat phase" is meant to include the oil present in the liquid state and forming a continuous phase as well as the solid fat particles contained in the liquid oil that have been phase-separated from the liquid oil by crystallization of fat by the plastification treatment. The "continuous fat phase" does not, however, include any fat contained in the dispersed aqueous phase, as occurs e.g., in a product  
25 having a so-called oil-in-water-in-oil structure.

The aqueous phase may comprise, apart from water, a gelling agent and, optionally, a thickener and/or one or more other ingredients that are commonly incorporated in margarines and spreads, e.g. flavouring agents, colouring agents, emulsifiers, salt,  
30 preservative and acid.

Similarly, additives may be contained in the fat phase composition. For example, the fat phase composition may comprise a blend of triglycerides supplemented with one or more emulsifiers and/or colouring agents.

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The aqueous phase composition may include some fat, but preferably the fat content of the aqueous phase composition is not higher than about 10 wt.%. It is particularly preferred that the aqueous phase composition comprises essentially no fat.

- 10     Throughout this specification the terms oil and fat are used interchangeably. They are meant to include triglycerides such as soyabean oil, sunflower oil, palm oil, fish oil, rapeseed oil, coconut oil, chemically and/or physically modified products such as hydrogenated, fractionated and/or interesterified triglyceride mixtures and mixtures of two or more thereof, as well as edible substances that are physically similar to
  - 15     triglycerides such as waxes, e.g. jojoba oil, and poly fatty acid esters of mono- or disaccharides, and that can be used as replacement for or in admixture with triglycerides. Preferably, the fat contained in the present spreads consists essentially of triglycerides.
  - 20     The aqueous phase and/or the fat phase can suitably include emulsifiers. The amount and kind of emulsifier included are not critical. It is preferred to incorporate emulsifiers of the type and quantity as are commonly used in spreads. For example, mixtures of mono- and diglycerides derived from natural, partially hydrogenated or fully hardened sunflower oil can suitably be employed, using an amount of about 0.2
  - 25     to about 0.5 wt.%, calculated on the total weight of aqueous phase and fat phase. Alternatively, other oil-compatible emulsifiers can be used. Mixtures of such emulsifiers with mono- and/or diglycerides can also be suitable as emulsifier.
- Typically, the average droplet size of the dispersed aqueous phase is such that a satisfactory flavour release in the mouth can be obtained, while the product can have

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an adequate microbiological stability. Droplet sizes may be between about 3  $\mu\text{m}$  and about 60  $\mu\text{m}$ , but it may be larger or smaller than that. The average droplet size, as referred to herein, is the volume weighted mean of the droplet size distribution. It can be determined with NMR following the procedure as described in J. Colloid and

- 5 Interface Science (1972), 10, 206 and (1983), 93, 521.

The average droplet size of the present spreads can be varied easily, by adjusting the conditions during the preparation.

- 10 The present spreads can suitably be used e.g. on bread as butter substitutes. However, they can also be suitable for use as complete spread on their own, for example when containing a cheese or vegetable or fruit flavours or pieces.

15 The following non-limiting examples illustrate the invention and do not limit its scope in any way. In the examples and throughout this specification, all percentages, parts and ratios are by weight unless indicated otherwise.

#### Examples

- 20 Reference is made in the examples to Figure 1.

Figure 1 shows the dose dependent contraction of rat aorta caused by phenylephrine and the inhibition of this effect by a pine needle extract of the invention.

- 25 Example 1

#### Pine needle extraction

- 100 g pine needles from *Pinus massoniana* (isocupressic acids (ICA) content 30 0.33wt%) were cleaned with water, cut into small pieces (3~4 cm) and put in a flask.

500 g hexane was added to the flask and heated under stirring to reflux (~ 60°C) for about 3 to 5h. The resulting pine needle solution was filtered through a Büchner funnel and the hexane removed using a rotary evaporator. The crude extract contains 8wt% of compounds of the isocupressic acid family. This extract was not used for  
5 further experiments.

The residue which was left after treatment with hexane was transferred to a flask and 500 ml demineralised water was added. The mixture was stirred at 100°C for about 3-5h. Then the extract was filtered through a Büchner funnel and concentrated to 150  
10 ml. To this extract 12.5g resin (Dowex Marathon A, Polysep Industrial Consultants) was added; the temperature was maintained at 50°C for 3h. After filtration through a Büchner funnel to remove the resin, the solution was dried in a rotary evaporator to produce the pine needle powder (ICA content 0.003wt%).

15 Example 2

Thoracic aortas were obtained from spontaneous hypertensive rats (SHR). The thoracic aorta is cut into rings of 4 to 6 mm in length and each ring is connected to a tension transducer in a thermostatically controlled and oxygenated organ bath  
20 containing modified Krebs-Henselheit buffer. The contractions of rings of aorta are recorded continuously under isotonic conditions. After equilibrating the tissues, a single dose of 1 $\mu$ M phenylephrine was given to sensitise the tissue, followed by washout. Hereafter, two cumulative dose response curves of phenylephrine were generated. The first dose response curve was obtained in the absence of an extract  
25 and served as a control curve. After thorough washing (7 times) the tissues were incubated with the pine needle extract for 1 hour. Following this incubation period, a second dose response curve was obtained in the presence of a concentrated form of the extract. The data were analysed taking the maximal response of the reference curve as a control.

Figure 1 shows that phenylephrine causes a dose dependent contraction of rat aorta (the upper curve in the Figure). After incubation with pine needle extract of Example 1, the contraction of rat aorta by phenylephrine is clearly inhibited (the lower curve in the Figure).

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### Example 3

The following is an example of a spread according to the invention. The spread can be prepared according to the procedure described in Example 14 of WO 97/18320.

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#### Fat Phase:

Fat Blend*	40	%
Hymono 7804 (emulsifier)	0.3	%
Colour (2% β-carotene)	<u>0.02</u>	%
15 Total	40.32	%

\*87:13 by weight sunflower oil and hardstock

#### Aqueous Phase (to pH 5.1):

20 Water	55.94	%
Pine needle extract (dry basis)	0.5	%
Skimmed Milk Powder	1.5	%
Gelatin (270 bloom)	1.5	%
25 Potassium Sorbate	0.15	%
Citric Acid Powder	<u>0.07</u>	%
Total	59.66	%

Example 4*Preparation of extracts*

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Two pine needle extracts were produced. The first extract (Extract 1) is a comparative example containing isocupressic acids. The second example (Extract 2) is a purified form of the first extract and is an example of a material according to the invention.

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Extract 1 was obtained as follows:

- 100 g pine needles from *Pinus massoniana* was cleaned, cut into small pieces (about 3 to 4 cm) and put into a flask
- 1 litre water was added
- The mixture was heated and maintained at reflux temperature (about 100°C) for 5h.
- The pine needle residue was removed from the mixture
- The pine needle extract (Extract 1) was obtained by removing water using a rotary evaporator.

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Extract 2 was obtained by further treating Extract 1 as follows:

- 200 g pine needle extract obtained above as Extract 2 (containing ~5-10% water) was mixed with 1.2 l demineralised water. This was allowed to stand in a water bath at 70 °C for half an hour to dissolve.
- The mixture was transported to a reaction vessel and stirred for 15 minutes at 70 °C.
- 40 g resin (Dowex 50 W) was added and stirring was continued for three hours at 50 °C, the mixture was then filtered. After the filtration, this step (i.e., addition of resin, stirring and filtering) was repeated.

- 0.8g of Norit SA4 active carbon was added.
- The resulting mixture was stirred for 1 hour while keeping the temperature at 85 °C.
- The resulting mixture was filtered 3 times through a Büchner filter (54; Ø185mm).
- Water was evaporated from the filtrate using a rotary evaporator to obtain Extract 2.

10 The general procedure for the analytical method for determining isocupressic acid (ICA) is as follows:

The sample is extracted with 50 mL methylene chloride for about three hours by means of a method based on Soxhlet extraction. After extraction, the methylene chloride is removed via rotary evaporation. After this step, heptadecanoic acid is 15 added as an internal standard. This component is used to be able to quantify in the end. The samples are then dissolved in a little methylene chloride and put on a SPE column containing 500 mg of aminopropyl sorbent, which has been conditioned with the appropriate solvents beforehand. After the sample has been put on the SPE column the possible present "neutral" components are eluted with a solvent 20 containing 9:1 by volume diether ether: methanol. After this clean-up step, the acids are eluted with the same solvent but containing an additional 1% of acetic acid. After removal of the solvent in a heating block, the sample is derivatized by means of 2 M diazomethane in ether (making methyl esters of the carboxylic acid groups) and by using MSTFA (N-methyl-N-trimethylsilyltrifluoroacetamide) for silylating any free 25 hydroxyl groups left. This derivatisation step is done in sequence. After derivatisation the sample is properly diluted using isoctane and 1 µl is injected into the GC-MS. The GC-MS uses a 30 m CP-Sil 5 column (DB-1) with an internal diameter of 0.25 mm to separate the components. Temperature programming is used: 100 C, 1 minute hold ramped to 200 C at 40 °C / min after which the temperature is ramped to 250 °C

at 2 °C /min. The helium carrier gas flow is kept constant at 1.0 mL/min (= +/- 68 kPa gauge pressure at 100 °C). Splitless injection is used and the temperature of the injector is kept constant at 250 °C. MS transfer line is also kept constant at 250 °C. The MS detector was set to EI mode with an ionization energy of 70 eV. The mass range collected was from 50 to 550 Da using an electron multiplier voltage of 1250 V.

*Blood pressure lowering effects of pine needle extracts:*

10 Reference is made in this example to Table 1.

Table 1 shows the effect of pine needle extract on

- the median effective concentration ( $EC_{50}$ ) of phenylephrine and the difference between DRC1 and DRC2
- maximal effect ( $E_{max}$ ) relative to phenylephrine; the lower the value the more effective the extract

Isolated aorta rings from spontaneously hypertensive rats (SHR rats) were used as a model for testing vasoactive effects. Thoracic aortas were obtained from the SHR rats. The thoracic aorta was cut into rings of 4 to 6 mm in length and each ring was then connected to a tension transducer in a thermostatically controlled and carbogentated organ bath containing modified Krebs-Henseleit buffer. The contractions of rings of aorta were recorded continuously under isotonic conditions. After equilibrating the tissues, a single dose of 1 $\mu$ M phenylephrine was given to sensitise the tissue, followed by washout. Phenylephrine is an  $\alpha$ -adenergic agonist, and is used in this experiment to induce contraction of the aorta rings. Hereafter, two cumulative dose response curves (DRC1 and DRC2) of phenylephrine were generated. DRC1 was obtained in the absence of an extract and served as a control curve, while DRC2 was obtained after incubation with 50  $\mu$ g/mL pine needle extract.

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30 After thorough washing the tissues were incubated with the pine needle extract for 5

minutes. Following this incubation period, a second dose response curve was obtained in the presence of a concentrated form of the extract. The data were analysed taking

- the median effective concentration ( $EC_{50}$ )
- 5 - maximal effect ( $E_{max}$ ) relative to phenylephrine

The following extracts were tested:

1. *Pinus massoniana* before Norit/Ion-exchange resin treatment (Extract 1)
- 10 2. *Pinus massoniana* after Norit/Ion-exchange resin treatment (Extract 2)
3. Comparative example: Extract according to US-B1-6,329,000 (Ji Ling)
4. Comparative example: Extract according to US 5,690,984 (Lim Jung Geun)
5. Comparative example: Ether extract according to US 5,607,971 (Al-Mahmoud Mohsen)
- 15 6. Comparative example: Methylene chloride extract according to US 5,607,971 (Al-Mahmoud Mohsen)
7. Comparative example: Methanol extract according to US 5,607,971 (Al-Mahmoud Mohsen)

Table 1

Extracts	logEC <sub>50</sub> control	logEC <sub>50</sub> extract	Difference logEC <sub>50</sub>	E <sub>max</sub>	ICA family <sup>1</sup> (%)	D- quinic acid (%)	Shikimic acid (%)	LMW sugars <sup>2</sup>
1	-7.35	-6.31	-1.04	54	0.30	9.8	19.8	26.0
2	-6.81	-6.08	-0.73	79	0.00	15.3	21.0	27.0
3	-7.25	-6.49	-0.76	53	0.02	4.0	17.0	23.0
4	-7.21	-6.88	-0.34	88				
5	-7.17	-6.43	-0.74	74	15.33	0.3	0.6	0.8
6	-7.36	-6.16	-1.20	76	13.68	0.2	0.0	0.4
7	-7.24	-6.47	-0.77	90	1.93	1.0	2.3	12.5

<sup>1</sup> ICA family consists of dihydroagathic acid, imbricatoloic acid, isocupressic acid, agathic acid, acetyl imbricatoloic acid and acetyl isocupressic acid.

5      <sup>2</sup>Low molecular weight sugars are monosaccharide units and include glucose, fructose and inositol structures.

After incubation with Extract 1 the contraction of the rat aorta by phenylephrine is clearly inhibited (Emax 54). Extract 2 shows after removal of isocupressic acids by  
10      Norit treatment and ion exchange still activity (Emax 79) similar to extracts 5, 6, and 7 which are high in isocupressic acids.

#### Example 5

##### 15      Preparation of margarine

Two margarines were produced under the same process conditions.

*Formulation*

Ingredients	Reference (%)	With pine needle extract (%)
<i>Fat phase</i>		
Soybean oil	62	62
Soybean oil 36-38	9	9
Soybean oil 45	6	6
Palm oil	4	4
Dinodan OT	0.6	0.6
β-carotene	0.02	0.02
Flavour type 2934	0.02	0.02
Subtotal	81.64	81.64
<i>Water phase</i>		
Pine needle extract	0	0.5
Potassium sorbate	0.15	0.15
Citric acid	0.07	0.07
Skimmed milk powder	0.25	0.25
Flavour type 2935	0.02	0.02
Salt	0.3	0.3
Water	17.57	17.07
Subtotal	18.36	18.36

Process conditions

5

The process line was set up in an AAC configuration. In this configuration the A-unit is a scraped surface heat exchanger and the C-unit is a pin mill (kneader). The volume of the C-unit was reduced to 1/3 and the numbers of the pins on the shaft of the kneader was reduced from 17 to 5. The temperatures of the product inlet, outlet and

10 the cooling medium were registered.

*Preparation of the water phase*

For preparation and pasteurizing of the water phase the mixing, dispersing and homogenizing device was used. This device had a 25 litre thermally controlled vessel 15 with a scraped anchor agitator. During dissolving/dispersing of the dry materials of the water phase in the water, the mixture is circulated continuously and temperature

of the water phase is monitored in-line in the mixing zone of the colloid mill. The mixture was pasteurized at 70 °C during 10 minutes. Afterwards the water phase was cooled down till approximately 55 °C without mixing.

5    *Preparation of the fat phase*

During stirring, all ingredients of the fat phase were molten under nitrogen atmosphere, and heated till about 80 °C. Afterwards, the fat phase was cooled down under continuous stirring till 55 °C.

10    *Emulsification*

The water phase was added slowly to the fat phase under intensive stirring and emulsified to a water-in-oil emulsion. During emulsification the emulsion was tempered at 55 °C for at least 30 minutes.

15    *Processing*

A 30 kg-batch size was employed. The mix was stirred for 15 minutes before pumping. After pumping, the line was allowed to run for 15 minutes before any collection of product. During processing, the mass flow was fixed at approximately 50 kg/h. At the indicated measuring points the temperature was measured. When a

20    stable situation was reached, 15 tubs were filled with approximately 125g margarine.

The following process parameters (temperatures in °C) were recorded:

	A <sub>1</sub> in	A <sub>1</sub> exit	C <sub>1</sub> in	C <sub>1</sub> exit	A <sub>2</sub> in	A <sub>2</sub> exit	Cool A <sub>1</sub>	Cool A <sub>2</sub>
Reference	42.7	10.4	7.7	11.3	14.7	14.5	-5.0	-0.5
With pine needle extract	43.4	10.5	7.7	11.4	14.6	14.3	-5.0	-0.6

All tubs were placed over night at 1~3 °C and judged by a small technical panel. The margarine products were evaluated visually on color, water release, gloss external structure, sticking to knife and spreadability. Subsequently, the products were evaluated on taste, mouthfeel and tailing properties. Besides sensorial evaluation,  
5 hardness and impedance were measured as an indication of the consistency of the table margarine.

The results of the product evaluation are given in table below. The spreads containing pine needle extract had a higher c-value for hardness than standard spreads. All  
10 samples spread easily with no apparent water loss. The spreads of the invention had, in addition to increased hardness, good spreadability, good structure, good tailing and good sticking to the knife.